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13. ABSTRACT *(Maximum 200 words)*

The research problem we have been investigating is the purification of the glycerphosphate oxidase from the terminal oxidase in bloodstream trypanosomes. African trypanosomiasis remains one of the major diseases in the world today, affecting both man and animals. In addition, it remains on the list of the major health problems facing mankind today, which includes AIDS, malaria, cancer and heart disease. The land mass of Africa, south of the Sahara, over which tsetse flies are distributed and is thus virtually devoid of cattle and is estimated to be about 4 million square miles. It has been estimated that if this area could be used, at least 125 million cattle could be raised, more than doubling the present cattle population in Africa.

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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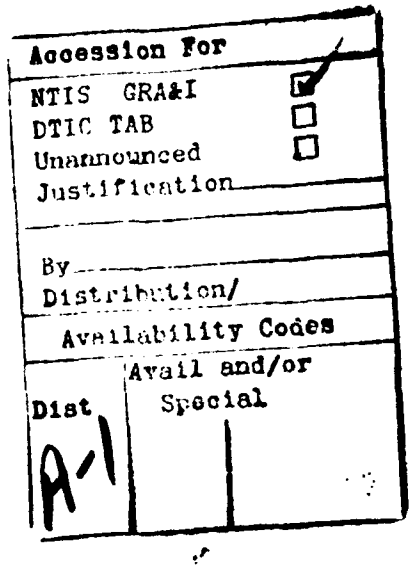

1. Statement of the Problem Under Study:

The research problem we have been investigating is the pruification of the glycerphosphate oxidase from the terminal oxidase in bloodstream trypanosomes. African trypanosomiasis remains one of the major diseases in the world today, affecting both man and animals. In addition, it remains on the list of the major health problems facing mankind today, which includes AIDS, malaria, cancer and heart disease. The land mass of Africa, south of the Sahara, over which tsetse flies are distributed and is thus virtually devoid of cattle and is estimated to be about 4 million square miles. It has been estimated that if this area could be used, at least 125 million cattle could be raised, more than doubling the present cattle population in Africa.

Experiments from a large number of investigators, including our laboratory, have demonstrated morphological, molecular and biochemical changes that occur as African trypanosomes undergo mitochondrial biogenesis (1-5,7). In the bloodstream trypomastigotes of Trypanosoma brucei, the mitochondrion is reduced to a single peripheral canal with few cristae. These organisms oxidize glucose to pyruvate which they cannot degrade further and re-oxidize NADH via the a-glycerophosphate oxidase system (GPO) which is insensitive to cyanide and sensitive to salicythydroxamic acid (SHAM). No cytochromes are present (1). When the organisms develop in the midgut of the tsetse fly as procyclic trypomastigotes, they have a complete electron transport system with two terminal oxidizes: cytochrome oxidase and probably GPO, although this has not been clearly shown (6). These organisms

undergo development into salivary gland metacyclic trypomastigotes which are infectious to the vertebrate host.

It is clear the GPO system is the only terminal oxidase in bloodstream forms (1). Figure 1 depicts the GPO within the inner membrane of the mitochondrion. The availability of the purified protein or even the N-terminal amino acid sequence would permit a wide range of studies on its expression, synthesis and control during differentiation. GPO, which is unique to the blood stream forms of trypanosomes, consists of two components: an FAD-containing sn-glycerol-3-phosphate dehydrogenase and an oxidase which are probably linked via ubiquinol (8). Studies using inhibitors of the GPO indicated that at least two enzyme components are present since the mitochondrial glycerol-3-phosphate dehydrogenase is capable of transferring electrons either to the SHAM-sensitive terminal oxidase component or to an artificial electron acceptor (9-10). Inhibitors acting after the site of reduction of the artificial electron acceptors were presumed to act on the terminal oxidase component (11).

The mitochondria of many fungi and higher plants have a branched electron transport system with a normal cytochrome chain and an alternative cyanide-resistant, hydroxamic acid-sensitive pathway (12). Electron transport in these systems branches at ubiquinone, and the alternative pathway is not linked to oxidative phosphorylation (13).

The alternative oxidase has been partially purified from several higher plants (14,15), but there is limited information about its identity in any organism. Initially, Elthon and McIntosh (16) have raised polyclonal antibodies to the solubilized and partially purified alternative oxidase from the S. guttatum. These antibodies immunoprecipitated alternative oxidase and have been used in immunoblotting experiments to identify three cross-reacting polypeptides with Mr of 37, 36, and 35 kD.

Subsequently, monoclonal antibodies produced by Elthon et al. (17) have now been prepared to the alternative oxidase from Sauromatum guttatum proteins and designated as AOA (binding all three proteins of the alternative oxidase cluster), AOU (binding the 37 kD protein), and AOL (binding the 36 and 35 kD proteins). All three antibodies bind to their respective alternative oxidase proteins whether the proteins are in their native or denatured states (as on protein blots). AOA and AOU inhibit alternative oxidase activity by approximately 49%, whereas AOL inhibits activity only 14%. When coupled individually to Sepharose 4B, all three monoclonal resins were capable of retaining the entire cluster of alternative oxidase proteins, suggesting that these proteins are physically associated in some manner. The monoclonals were capable of binding similar mitochondrial proteins in a number of thermogenic and nonthermogenic species, indicating that they will be useful in characterizing and purifying the alternative oxidase of different systems.

The ability of the monoclonal-Sepharose 4B resins to retain the cluster of previously identified alternative oxidase proteins, along with the inhibition of alternative oxidase

activity by these monoclonals, supports the role of these proteins in constituting the S. guttatum alternative oxidase. Lambowitz et al. (18) have used monoclonal antibody to the alternative oxidase of the higher plant S. guttatum to identify a similar set of related polypeptides (Mr 36 and 37 kD) that was associated with the alternative oxidase activity of N. crassa mitochondria. These polypeptides were not present constitutively in the mitochondria of a wild-type N. crassa strain, but were produced in high amounts under conditions that induced alternative oxidase activity.

Little information is known concerning the biochemical properties of the GPO. Recently, Clarkson et al. (19) have confirmed the presence of CoQ_9 by Hill and co-workers (20) and demonstrated important similarities between GPO and plant alternative oxidizes. The availability of alternate electron donors that we have identified for the GPO as well as the ability to solubilize this enzyme should facilitate the purification of the GPO, preparation of monoclonal antibodies for it, and the characterization of some of its important properties dealing with electron transfer which could be a target for inhibition. During differentiation, while SHAM sensitive activity is present in procyclic trypomastigotes, it has only been assumed this is GPO. If it is present, what are the control mechanisms involved in its regulation?

2. Background and Review of Appropriate Literature and/or Earlier Report:

Trypanosomes are flagellated protozoa responsible for several serious parasitic diseases of humans and domestic animals. Trypanosomes present various medical and economic obstacles for the development of several African and South American countries. African trypanosomiasis is ranked among the top six tropical diseases selected for scientific studies by the World Health Organization (21). These parasites are of interest to scientists not only because of their medical and veterinary importance but also because of several unique biochemical features. Bloodstream forms of T. brucei and T. rhodesiense lack cytochromes (11, 22), are completely dependent on glycolysis for energy, and respiration in whole cells is insensitive to inhibitors such as cyanide, azide, and antimycin A (22).

Early studies (23) on the respiratory system of trypanosomes indicated the presence of a distinct type of respiratory enzyme system in the bloodstream form of Kinetoplastida. von Brand et al. (24) also observed that the respiration of various bloodstream forms of trypanosomes was not inhibited but stimulated by KCN, thereby suggesting the presence of an alternative respiratory system.

Grant and Sargent (25) were the first to observe a particulate L-a-glycerophosphate oxidase int he bloodstream form of T. rhodesiense, which would react with oxygen without the presence of either pyridine nucleotide enzymes or cytochromes. Fairlamb and Bowman

(8) also suggested that during glycolysis the reoxidation of NADH was generated by means of an NAD dependent glycerol-3-phosphate dehydrogenase, which catalyzes the formation of glycerol-3-phosphate from dihydroxyacetone phosphate. Glycerol-3-phosphate is then reoxidized to the dihydroxyacetone phosphate by the mitochondrial glycerol-3-phosphate oxidase (GPO).

Like most cyanide insensitive electron transport systems which are found in certain plants and fungal mitochondria (26), the GPO system can be completely inhibited by hydroxamic acids such as salicylhydroxamic acids (SHAM) (27, 19). Hill (1) has shown that GPO has a lower affinity for oxygen than cytochrome oxidase (0.1 versus 2.1 M) but it apparently reduces oxygen to water as the end product, since hydrogen peroxide could not be detected as a free intermediate (28).

GPO, which is unique to the bloodstream forms of trypanosomes consist of two components: a flavin-linked glycerol-3-phosphate dehydrogenase (E.C.1.1.99.5) and an oxidase which are probably linked via ubiquinol (8). Studies using inhibitors of the GPO indicated that at least two enzyme components are present. Since the mitochondrial glycerol-3-phosphate dehydrogenase is capable of transferring electrons either to the SHAM-sensitive terminal oxidase component or to an artificial electron acceptor (8, 9, 29). Inhibitors acting after the site of reduction of the artificial electron acceptors were presumed to act on the terminal oxidase component (11). The oxidase component deserves further investigation, as it is the enzyme which is absent in the mammalian host and is specifically

inhibited by SHAM. This enzyme is therefore of interest as a possible target for drug chemotherapy. At present only suramin and organic arsenicals remain as the mainstay of chemotherapy, despite their many dangerous disadvantages.

With the use of a fast protein liquid chromatography (FPLC) system and a Mono Q anion exchange column, we have been able to solubilize and partially purify the GPO activity.

3. Rationale Used in Current Study:

The GPO is the only terminal oxidase functioning in bloodstream trypomastigotes. This oxidase has not been purified or characterized. Whether the same alternative oxidase is present in procyclic forms remains to be determined. These studies will be valuable in providing biochemical information which could contribute to understanding the regulation of the GPO gene and characterization of the biochemical properties of this alternative oxidase.

4. Experimental Methods:

Isolation of trypanosomes. Trypanosoma brucei EATRO 110 was obtained from Dr.

M. R. Rifkin at Rockefeller University in New York. This strain was isolated in 1958 in East Africa from a naturally infected bovine and it has been cyclically passaged in tsetse flies and rodents. Male Sprague-Dawley (250-300 g) rats were inoculated intraperitoneally with $1x10^7$ cells/100 g weight. The animals were killed when the parasitemia level was between $8x10^8$ and $1x10^9$ cells/ml blood. The rats were anesthetized with ether and bled to death. A buffy coat was prepared and the trypanosomes were separated from the red blood cells on a DEAE cellulose column (30) eluted with phosphate buffered saline with glucose and heparin (PBSGH) at room temperature.

Preparation of parasite mitochondria. Parasites were pellete ' by centrifugation in either a Sorvall HS-4 or GS-3 rotor for 5 minutes at 4,000 x g at 4°C. The pellet was swollen in 1 mM Tris and 1 mM EDTA pH 8.0, nomogenized with a Teflon fitted hand homogenizer, and then passed three times through a 26 G 1/2" needle with 80 psi. Osmolarity was restored with the addition of sucrose, EDTA, and 0.5 M Tris, pH 7.5. Crude mitochondria was collected after centrifugation at 27,000 X g for 10 minutes. The pellet was then treated with 10 ug/ml DNAse I for 30 minutes at 4°C. Mitochondria were isolated on a 20-35% renografin gradient as described by Braly et al. (31).

Effect of lauryl maltoside on the release of GPO. Frozen mitochondria were thawed and exposed to various concentrations (1-15 mM) of lauryl maltoside for 8 hr. The samples were centrifuged at 48,000 x g for 20 minutes at 4°C and the supernatant fluids were assayed

for enzyme activity. Control experiments consisted of exposing the mitochondria in buffer without detergent for the same amount of time followed by centrifugation at 48,000 X g for 20 minutes. Experiments with centrifugation at 100,000 x g yielded similar results.

Enzyme purification. Five mg of trypanosomal mitochondrial protein were exposed to 1 ml of loading buffer (7.5 mM lauryl maltoside, 10 mM K_2HPO_4, 5% sucrose, and 210 mM KCl, pH 7.5) for 2 hr at 4°C. The Mono Q HR 5/5 anion exchange column (1.0 ml bed volume) was equilibrated with 5 ml running buffer A (7.5 mM lauryl maltoside, 10 mM K_2HPO_4, 5% sucrose and 1M KCl, pH 7.5) then again with 5 ml running buffer A. Two mg of lauryl maltoside solubilized mitochondrial protein were loaded onto the anion exchange column and eluted with a 50 ml linear KCl gradient (0 to 1 M) at a flow rate of 0.5 ml/min. The effluent was monitored at 280 nm in a FPLC UV absorbance detector set at 0.5 full scale deflection and 1 ml fractions were collected. All fractions were assayed for protein concentrations using the dye-binding method described by Bradford (32).

Enzyme assay. Protein fractions eluted from the FPLC system were measured for enzyme activity with a Instech 125/05 oxygen electrode maintained at 25°C by a circulating water bath. Oxygen consumption was measured by a Yellow Spring Model 5300 oxygen monitor. The oxygen electrode chamber contained 0.5 ml of 40 mM Tris, pH 8.0 and 3 mg/ml bovine serum albumin. Oxidase activity was measured after the addition of 0.6 mM of the ubiquinol analog, 2,3,-dimethoxy-5-methyl-6-nonyl-1,4-benzoquinone which was

synthesized according to Catlin et al. (33) as the electron donor. To confirm the oxidase activity, 0.5 mM SHAM was used to inhibit the oxidase activity 100%.

5. Results Presented as Figures, Tables and Narrative Descriptions:

Effect of lauryl maltoside on GPO. Figures 2 and 3 show the amount of GPO released from the mitochondria membrane after treatment with lauryl maltoside. The highest amount of activity released from the mitochondria membrane occurred after exposure to a concentration of 7.5 mM lauryl maltoside for four hours. After 2 hours of exposure to the detergent, specific activity for the oxidase was observed to be 24 nmoles 0_2 consumed/min/mg protein. Exposure to the detergent lauryl maltoside after 4 hours increased the specific activity to 28 nmoles 0_2 consumed/min/mg protein. However, it was also observed that after 4-8 hours of exposure (fig. 4), enzyme activity for the oxidase decreased 75%. No enzyme activity was detected in the supernatant fluids of the controls indicating that the activity measured was released from the mitochondria after detergent treatment.

Enzyme purification. A summary of the partial purification of the ubiquinol oxidase is shown in Table 1. This procedure resulted in an overall recovery of 13.4% and a 22.4 fold purification. Eighty percent of the protein was recovered from the column with 60% of the protein eluted in the void volume. The oxidase was eluted from the column between

300 and 400 mM KCl. Fractions containing the enzyme were totally inhibited by the addition of SHAM to the reaction mixture.

Enzyme assays. The elution profile of the oxidase is shown in figure 5. All fractions containing proteins were assayed for oxidase activity. The first five fraction that contained 60% (0.8-1.2 mg) of the loaded protein were negative for the oxidase. Proteins eluted from the anion exchange column between 300 and 400 mM KCl (fractions 19, 20, and 21) contained 30.0, 30.0 and 10.0 ug of protein, respectively (fig. 4). No enzyme activity was detected in fraction 19. The specific activity of fraction 20 was 560 nmoles 0_2 consumed/min/mg protein and that of fraction 21 was 420 nmoles 0_2 consumed/min/mg protein. Enzyme activity in both fractions were inhibited 100% by the addition of 0.5 mM SHAM.

6. Discussion and Conclusion:

Partial purification of GPO can be obtained with the use of a FPLC system and a Mono Q anion exchange column. After the addition of 2 mg of solubilized mitochondrial proteins with specific activity of 16 nmoles 0_2 consumed/min/mg protein were applied to the anion exchange column, the oxidase was eluted between 300 and 400 mM KCl. Fractions containing 30 ug of protein had a specific activity of 560 nmoles 0_2 consumed/min/mg protein for the oxidase resulting in a 22.4 fold enrichment for the

oxidase. These results also indicate that frozen mitochondria can be used for these experiments with success. Apparently prolonged freezing is not detrimental to the oxidase. However, if the oxidase is stored overnight at 4°C, enzyme activity can not be detected. This indicates that the oxidase is unstable once removed from the mitochondrial membrane.

The major problem encountered with this purification scheme is the initial loss of activity after centrifugation. Approximately 60% of the enzyme is pelleted with mitochondrial membranes, clearly revealing that the detergent does not lyse the mitochondria completely. This occurs as a result of selecting a mild but effective detergent which is effective in releasing hydrophobic proteins. One advantage of using this purification scheme is that a small amount of material is required. Therefore the use of trypanosomal mitochondria is practical, for with 1 mg of solubilized mitochondrial protein the FPLC monitor can detect the protein peak containing the oxidase activity.

This is the first successful report on the partial purification of the oxidase from the mitochondrial membrane of T. brucei. Tielens and Hill (35) reported that with the aid of affinity chromatography they were able to bind a fraction of T. brucei protein to the affinity column and that the oxidase was eluted from the column with borate but the oxidase activity could not be detected. Fairlamb and Bowman (8) were able to partially purify GPO 24 fold from lysed T. brucei with a combination of differential and isopycnic sucrose gradient centrifugation, but since no detergent was used the enzyme was probably still bound to the membrane.

Enzyme assays for GPO have shown that the oxidase is a part of GPO. Enzyme activity of the oxidase was detected in the holoenzyme with the addition of a ubiquinol analog or with the substrate glycerol-3-phosphate. However, when the holoenzyme is solubilized the oxidase activity can only be measured with the addition of the ubiquinol analog. This suggests that the oxidation of glycerol-3-phosphate and ubiquinol analog are not two independent processes but are reactions of the same enzyme complex. The purification of the oxidase from the mitochondrial membrane of T. brucei supports the studies of Opperdoes and Borst (34) who suggested that the cyanide insensitive respiratory chain is localized in the mitochondria and is probably the main enzyme system responsible for reoxidizing the NADH generated in aerobic glycolysis. The NADH is reoxidized by a NAD-dependent glycerol-3-phosphate dehydrogenase and then the reducing equivalents are oxidized by the cyanide-insensitive glycerol-3-phosphate oxidase located in the mitochondria as described by Fairlamb and Bowman (9). The electron carrier between the two enzymes is unknown. However, it appears to be a ubiquinone-like mediator which aids in the transfer of electrons between the two enzymes. This is supported by the use of ubiquinol analogues which restore the oxidase activity after the GPO complex has been solubilized with detergents. Similar observations were reported by Tielens and Hill (35), who used ubiquinol analog to measure the oxidase activity of GPO from mitochondria treated with octylglucoside.

Lauryl maltoside is similar to octylglucoside except for the presence of an alkyl side chain. This detergent has been used to solubilize cytochrome oxidase from mitochondria

of other organisms (36). It was observed that optimum release of the enzyme occurred at a concentration of 7.5 mM detergent after four hours of exposure. Tielens and Hill (1) observed that the optimal concentration for octylglucoside was 2% and 1% for deoxycholate which resulted in a 30-60% recovery of enzyme activity. However, it is interesting that prolonged exposure to a 1mM concentration of lauryl maltoside caused a decrease in enzyme activity. Another disadvantage of the detergent lauryl maltoside is that it does not completely separate the holoenzyme for there is some activity of the GPO complex after 4 hr at a concentration of 15 mM. Tielens and Hill (35) reported that after samples were treated with octylglucoside no activity for the holoenzyme could be detected.

The results of the enzyme purification indicates that after the mitochondria had been solubilized with 7.5 mM lauryl maltoside, a 30 fold enrichment of the oxidase could be obtained. Several investigators (8, 35) have isolated either the oxidase or the dehydrogenase from trypanosomes using conventional protein purification methods. When enzymes assays were carried out on lysed whole cells and treated with various concentration of lauryl maltoside the oxidase was inhibited 98-100%. Concentrations as low as 1mM lauryl maltoside inhibited enzyme activity approximately 98%, suggesting the release of proteins which probably either block or degrade the oxidase activity. The results presented indicate that this procedure can serve as a preliminary step for the purification of the oxidase. Purification of the enzyme will provide a protein complex available for inhibition studies. Current studies are devoted to the identification of proteins bands associated with the enzyme activity.

REFERENCES

1. Hill, G. C. (1976) Biochem. Biophys. Acta 456,149-193.

2. Bienen, E.J., Hammadi, E. and Hill, G.C. (1980) J Parasit. 66, 680-682.

3. Bienen, E.J., Hammadi, E. and Hill, G.C. (1981) Exper. Parasit. 51, 408-417.

4. Bienen, E. J., Hill, G.C. and Shin, K.O. (1983) Mol. Biochem. Parasit. 7, 75-86.

5. Englund, P.T., Hajduk, S.L. and Marini, J.C. (1982) Ann. Rev. Biochem. 51, 695-726.

6. Grivell, L.A. (1983) Scientific American 248, 78-89.

7. Simpson, L. (1986) Int. Rev. Cytol. 99, 119-179.

8. Fairlamb, A.H. and Bowman, I.B.R. (1977) Int. J. Biochem. 8, 669-675.

9. Fairlamb, A.H. and Bowman, I.B.R. (1977) Int. J. Biochem. 8, 659-668.

10. LeFebvre, R.B. and Hill, G.C. (1986) Exper. Parasit. 62, 85-91.

11. Fairlamb, A.H. and Opperdoes, F.P. (1986) Plenum Publishing Corporation, New York City.

12. Henry, M.F. and Nyns, E.J. (1975) Sub. Cell Biochem. 4, 1-65.

13. Palmer, J.M. (1976) Ann. Rev. Plant Physiol. 27, 133-157.

14. Sanger, F., Mikien, S., and Coulson, A.R. (1977) Natl. Acad. Sci. Proceed. 74: 5463-5467.

15. Pettigrew, G.W. (1979) in Levandowsky, M., and Hutner, S.H., Eds.: New York: *Academic Press: p.* 59.

16. Elthon, T.E., and McIntosh, L. (1986) Plant Physiol. 82, 1-6.

17. Elthon, T.E., Nickels, R.L. and McIntosh, L (1989) Plant Physiol. 89, 1311-1317.

18. Lambowitz, A.M., Sabourin, J.R., Bertrand, H., Nickels, R., and McIntosh L. (1989) Mol. Cell Bio. 9, 1362-1364.

19. Clarkson, A.B., Bienen, E.J., Pollakis, G. and R.W. Grady, (1981) J. Biol. Chem. 264, 17770-17776.

20. Folkers, K., Vadhanavikit, S., Bueding, E., Hill, G. and Whittaker, C. (1983) Chem.-Ztg. 107, 131-133.

21. Trigg, P.I. 1979. Trends Biochem. Sci. 4, 29-30.

22. Flynn, I.W. and Bowman, I.B.R. 1973. Comp. Biochem. Physiol. 45B, 25-42.

23. von Brand, T. and Tobie, E.J. 1948. J. Cell Comp. Physiol. 31, 49-68.

24. von Brand, T. and Tobie E. J. and Mehlman, B. 1950. J. Cell. Comp. Physiol. 35, 273-300.

25. Grant, P.T. and Sargent 1961. Biochem. J. 81, 204-214.

26. Henry, M.F. and Nyns, E.J. 1975. Sub-Cell Biochem. 4, 1-65.

27. Opperdoes, F.R., Borst, P. and Fonck, K. 1976. FEBS Letters 62, 169-172.

28. Caughey, B., Hill, G.C. and Rich, Pl 1979. J. Protozool. 26, 8A.

29. Meshnick, S.R., Blobstein, S.H., Grady, R.W. and Cerami, A. 1978. J. Exp. Med. 148, 569-579.

30. Lanham, S.M. 1968. Nature (Lond). 218, 1273-1274.

31. Braly, P., Simpson, L. and Kretzer, F. 1971. J. Protozool. 21, 782.

32. Bradford, M.M. 1976. Anal. Biochem. 72, 248-254.

33. Catlin, J.C., Daves, C.D., and Folkers, K. 1971. J. Med. Chem. 14, 45-48.

34. Opperdoes, F.R. and Borst, P. 1977. Localization of nine glycolytic enzymes in a microbody-like organelle in Trypanosoma brucei: The glycosome. FEBS Letters 80, 360-364.

35. Tielens, A.G.M. and Hill, G.C. 1985. J. Parasit. 71, 384-386.

36. Dutch, R., Ingle, R., Centeno, J., Peiffer, W., Babcock, G. and Ferguson-Miller, S. 1987. Plant Mitochondria (Moore, A.L. and Beechery, R.B., ed) pp. 301-304, Plenum Publishing Corporation, New York City.

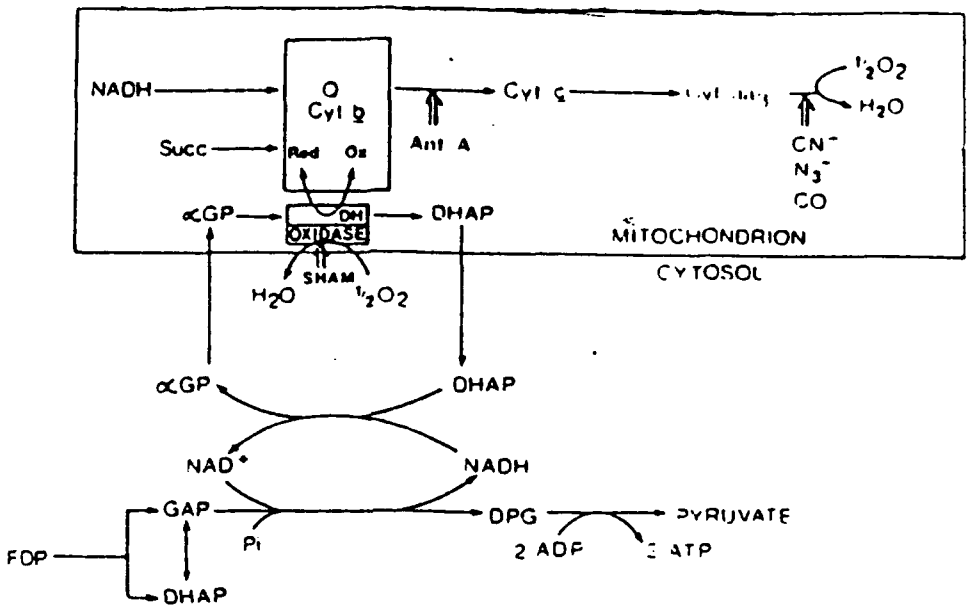

Fig. 1. The proposed arrangement of respiratory systems in the procyclic trypomastigotes of Trypanosoma brucei. The abbreviations used are Succ., succinate: Red and Ox unidentified component that can transfer electrons between the GPO and cytochrome system.

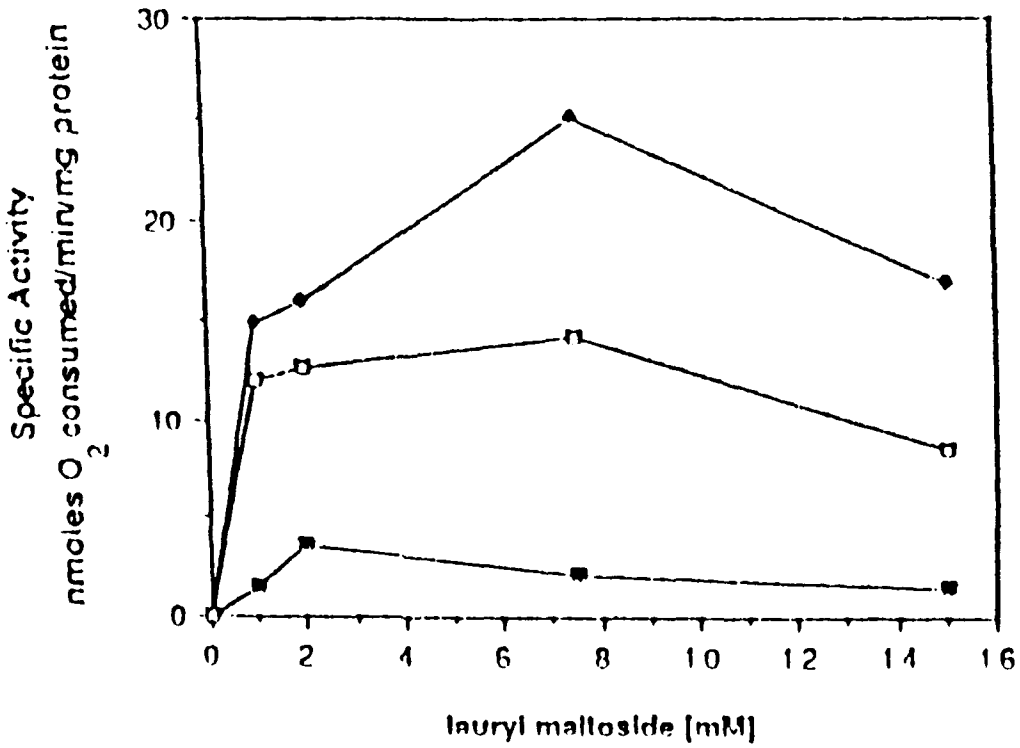

Fig. 2. The effect of various concentrations of lauryl maltoside on the release of enzyme activity from the mitochondria of T. brucei after 2 hrs. of detergent treatment. Enzyme assays were on the supernatant after a 48,000 X g centrifugation for 10 min. No enzyme activity was observed in the controls, (■) GPO, (♦) UBQO, (□) G-3PDH.

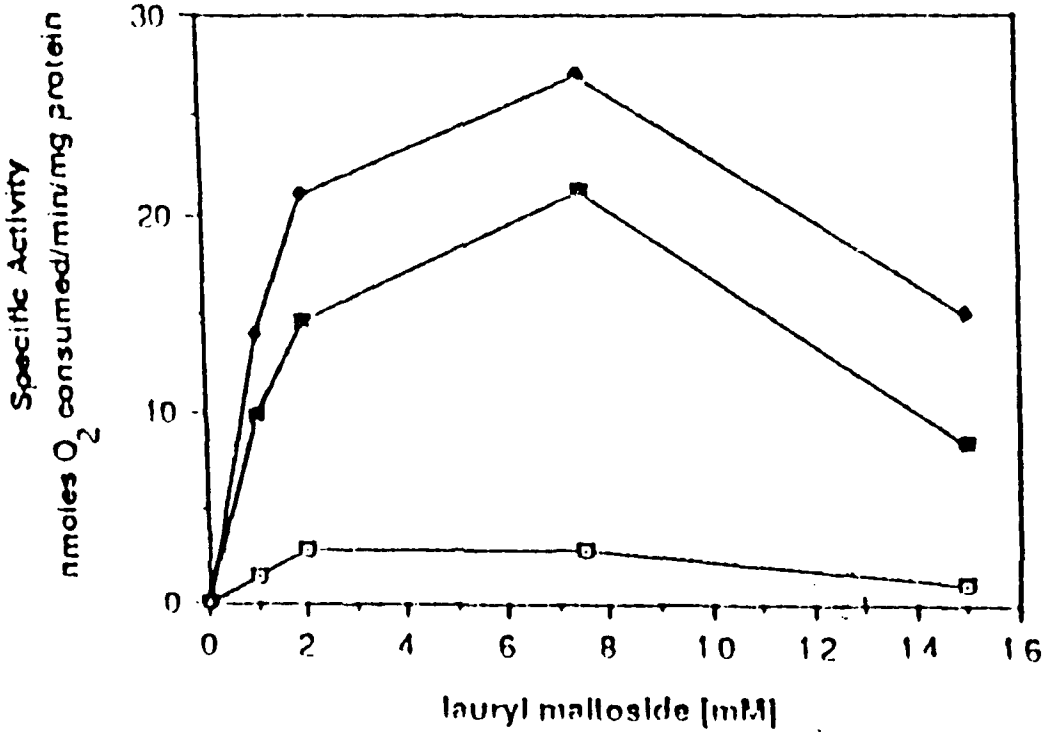

Fig. 3. The effect of various concentrations of lauryl maltoside on the release of T. brucei enzyme activity after 4 hrs. of exposure to the detergent. (□) GPO, (♦) UBQO, (■) G-3PDH.

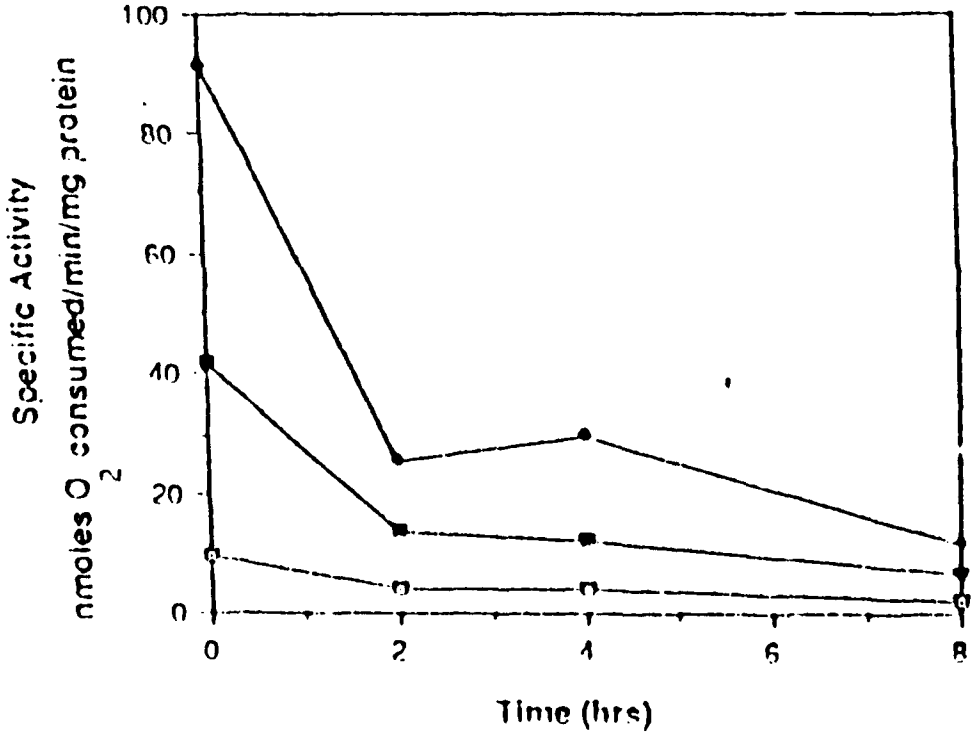

Fig. 4. A time course of T. brucei enzyme activity after exposure to 7.5 mM lauryl maltoside for 8 hrs. (□) GPO, (◆) UBQO, (■) G-3PDH.

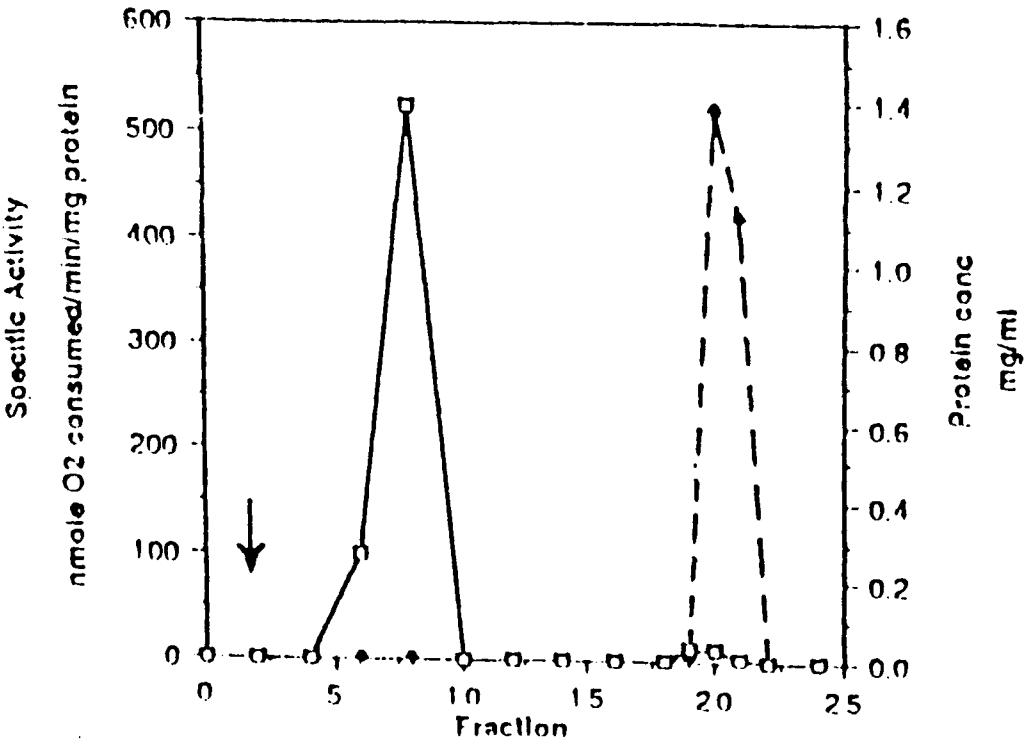

Fig. 5. Elution profile of proteins and ubiquinol oxdase activity (UBQO) from solubilized T. brucei mitochondria after FPLC anion exchange chromatography. The enzyme was eluted as described in the text. The arrow indicates the addition of 2 mg of solubilized mitochondria protein on to the Mono Q HR 5/5 anion exchange column (–■–) protein, (--◆--) UBQO.

Table 1. Solubilization and partial purification of ubiquinol oxidase from Trypanosoma brucei

	Total Protein (mg)	Specific activity nmoles O_2/min/ mg protein	Enzyme activity nmoles O_2/ min	Purification	Percent Yield
Mitochondria	5.0	25.0	125.0	1.0	100.0
Solubilized Mitochondria Protein*	2.0	16.0	32.0	0.64	25.0
Mono Q**	0.03	560.0	16.8	22.4	13.4

* Protein released as a result of 7.5 mM lauryl maltoside.
** Pharmacia anion exchange column.